OPTICAL PROPERTIES OF VIRUSES

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LONG-TERM GOALS

The long-range goal of this ONR-sponsored research is to document the importance of viruses to light scattering in the sea.

OBJECTIVES

The objectives of this work are to: 1)understand the optical properties of some common viruses, 2) examine the optical impact of several common marine bacteriophages, and 3) assess how important viruses are to the total absorption, scattering and backscattering of dissolved organic matter in sea water. Such results will be directly applicable to bio-optical models.

APPROACH

The first phase of this project has been to understand the optical properties of some common viruses. The rationale behind beginning with these types of viruses is that they are easily propagated, they can be grown in large concentrations, and they are simple to enumerate. Viruses are propagated on bacterial host cultures. Size distribution of the viral suspensions has been quantified using flow-field fractionation. A Wyatt Technologies Light scattering photometer has been used to estimate the volume scattering of these particles.

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Many experiments have been performed in which two types of phage have been propagated to concentrations of 108 to 1010 viruses mL-1. These phages are dramatically different in size and morphology.

Propagation, Purification and Enumeration of Viruses

Bacteriophage types and their appropriate host cell were obtained from the American Type Culture Collection (ATCC, Bethesda, MD). The first viral strain tested was MS-2, ATCC 15597 B-1 (host cell, E. coli ATCC #15597), which is a small icosohedral virus (25-30nm) containing single-stranded RNA. The second phage to be used was T-4, ATCC 11303 B-1 (host cell, E. coli ATCC #11303), a 225 nm double-stranded DNA phage possessing an elongated head and tail structure. E. coli host stocks were cultured in tryptone broth. Short term storage of host cultures involved the preparation of cultures on tryptone agar slants. Following inoculation, cultures were incubated (36 C) overnight. Slants could be stored at 4ºC for up to two weeks. For long term storage, overnight broth cultures were supplemented with 10% sterile glycerol, dispensed into 1 ml aliquots in cryovials, and stored frozen at -74ºC. Bacteriophage MS-2 and T-4 stocks were prepared in a similar manner. Overnight cultures of respective host cells were inoculated into tryptone broth and incubated with shaking for 2 hr. One-ml volumes of appropriate phage stock were then added, and the incubation continued for an additional 4 hr. Harvested lysates were centrifuged (5000xg/15 min) to remove debris, and further clarified via filtration (0.45 um). Resulting bacteriophage titers, enumerated by a plaque assay method (Adams 1959), averaged 10^{11}pfu/ml for MS-2, and10^{10} pfu/ml for T-4. Phage stocks were stored in 1 ml aliquots at -74ºC.

Purified, high titer phage stocks used in optical measurement experiments were prepared in the following manner. Overnight host cell cultures were inoculated into 1 L volumes of tryptone broth and incubated with shaking for 2 hr. Cultures were then inoculated with 10^{10} to 10^{11} pfu of appropriate bacteriophage and incubation continued for 4 hr. Lysates were then centrifuged and filtered as described above, and the phage concentrated by ultracentrifugation (230,000xg/2 hr). Resulting pellets were resuspended in 10 ml of phosphate buffered saline (PBS). Concentrates were layered onto cesium chloride gradients (1.3 - 1.7 g/ml) and centrifuged (100,000 xg/60 min/10C), as per Bachrach and Freidman (1971). Resulting virus bands were collected and dialyzed overnight (10ºC) against 1/2 strength PBS. Purified, concentrated virus stocks were assayed and stored at 4ºC until used. Stocks were usually prepared several days prior to each experiment. None were stored for more that 5 days before use. Phage concentrations in purified stocks averaged 10^{13} - 10^{14} pfu/ml for MS-2 and 10^{12} - 10^{13} pfu/ml for T-4.

Size Distribution Measurements

For calculation of absorption, scattering and backscattering efficiencies, one needs to know the optical as well as physical cross-sections of the particles. Due to the small size of viral particles, Coulter counting cannot be effectively used to estimate the size spectrum of a viral suspension. The most effective means to acquire such a size spectrum is with field-flow fractionation (FFF; examples of this technique applied to aqueous particle suspensions can be found in Ratanathanawongs and Giddings (1991), Beckett and Hart, (1993), Wahlund and Litzen (1989)). This is a chromatographic-like separation which

occurs in a thin ribbon-shaped channel as an external field and/or pressure gradient is applied perpendicularly to the flow channel. Typically used fields or gradients for fractionation are sedimentation, thermal or cross-flow; FFF allows fractionation of materials from a few thousand molecular weight to 100mm particle diameters. FFF has been used previously to size fractionate viruses (Giddings et al, 1980). In the context of these experiments, FFF allows separation of viral aggregates and membrane fragments from solitary viruses for subsequent optical measurements.

Optical Measurements

A Dawn Laser Light Scattering Photometer has been used for measurement of the volume scattering function, and calculation of the backscattering coefficient (bb). This instrument makes 400 measurements of the phase function each second and averages the data over any pre-set time period. WMB currently owns a Wyatt Technologies Dawn Photometer equipped with argon ion laser light source (512 nm) with capability of discrete or continuous flow-through measurements. As part of this work, the instrument was upgraded from a helium neon laser. The upgrade provides light at a wavelength more relevant to the ocean. Light scattering measurements can be directly coupled to size measurements from the field-flow fractionator. Thus, for each size fraction separated by the field-flow fractionator, we produced an average volume scattering function and bb estimate.

Next, optical absorption was determined on aliquots of purified virus. Viral suspensions were placed in a quartz cuvette, for absorption measurements using the technique of Bricaud et al. (1983). The cuvette containing the purified viruses in buffer is placed close to the photodetector of a Optimetrics Cam Spec M330 spectrophotometer for an absorption measurement. Such measurements have been particularly useful for defining the absorption properties in the near-UV part of the spectrum. It is important to point out that we have used high viral titers in order to maximize the signal to noise ratio during optical measurements.

Effects of Virus Concentration on Optical Properties

Following each optical measurement, the viral cultures were serially diluted and re-assayed to verify the concentration dependence of viruses on their associated optical properties. Observations also were made to determine the impact of phage on the optical properties of the host bacteria, before and after viral attachment. Initial experiments were conducted in phosphate buffered saline (pH 7.2). Once absorption, scattering, and attenuation characteristics were established, serial dilution experiments were repeated in ultra-filtered sea water from the Gulf of Maine into which a known titer of the above viruses was placed. This last step, while not the same as adding marine viruses to sea water, provides a preliminary examination of viral optical properties in sea water.

RESULTS

Our results have quantified the backscattering cross section for the two above-named viruses. Serial dilution experiments demonstrated a viral impact on backscattering at the upper-end of the concentration range found in seawater,in addition, the linearity of backscattering versus dilution experiments was strong. The shape of the volume

scattering function was quite different for the two viral types. It should also be noted that the backscattering cross sections were significantly higher than published values which were based on Mie scattering calculations. Moreover, viral aggregates were not an issue given that flow field fractionation demonstrated viral particles to be mostly solitary (i.e. the particle spectrum for our two viral suspensions shows a mode at the diameter of the viruses based on scanning electron micrographs).

IMPACT/APPLICATIONS

These results are of use in understanding the overall role of viruses in the sea. At present, we would concur with others that viruses, while a significant source of backscattering, are not the most important source of backscattering in the sea. Nevertheless, our calculated backscattering cross sections are higher than those predicted from theory. Thus, their impact should not be underestimated, particularly in coastal, polluted areas. One final application of this work is that we now have a means to optically measure viral titer of a solution. This has many potential medical applications.

TRANSITIONS

We are examining the impact of viruses on bacterial volume scattering. Moreover, we are performing intentional bacterial infection experiments to understand the effect of these processes on the particle optical properties. We now are propagating some true marine viruses to examine their optical properties.

RELATED PROJECTS

This DEPSCoR work is a joint venture between Bigelow Laboratory and their affiliate, the University of New England. Collaborative relationships are maintained with Dr. Ken Voss and Dr. Howard Gordon, both ONR-funded investigators at the University of Miami Dept. of Physics.

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